Protective effect of single or combined treatment of *Moringa oleifera* and *Citrullus colocynthis* on carbon tetrachloride induced hematological and cardiac toxicity in rats

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**ABSTRACT**

*Moringa oleifera* and *Citrullus colocynthis* have previously been reported to have protective effect on hepatorenal function in experimental model of toxicity. To better understand the effect of single or combined treatment of tested compounds of raw extract of both plants on hematotoxicity and cardiomyopathy induced by carbon tetrachloride in male albino rats, we used 56 male albino rats which were divided into 7 groups. First group served as a control, while second one was considered as control positive of cardiomyopathy induced by carbon tetrachloride at dose of 1 ml/kg. Third to sixth groups were treated with carbon tetrachloride beside plants treatment for eight weeks. The seven groups received both cardiomyopathy agents, 250 mg/kg of *M. oleifera* and 12.5 mg of *C. colocynthis*. It was found that different doses of single or combined treatment of ethanolic extract of both plants reduced the levels of lipid profile when compared with the control positive. Hematological, Histopathological and Electrophysiological (ECG) Assessment of Cardiac indices proof that tested compounds of raw extracts retain heart colorless liquid that is widely used as Chemical compound for producing a model of tissue Architecture. Notably, plants treatments have antioxidant effects evidenced by reduction level of collagen deposition and inflammation in heart recorded by Mallory trichrome staining and confirmed by detection levels of smooth muscle actin and il-6. Therefore, both plants could be a source of therapeutics of cardiomyopathy and hematological improvements in many diseases.

**Key words:** *Moringa oleifera*, *Citrullus colocynthis*, heart indices, lipid profile, ccl4-induced injury, albino rats.

**INTRODUCTION**

Carbon tetrachloride (CCl4) is a volatile, nonflammable and toxicity (Adaramoye, 2009) in different organs such as liver, heart, blood, kidney and brain (Ozturk et al., 2003). The high dose of ccl4 rapidly causes oxidative stress, inflammation and cellular necrosis that causes injury of the tissue or failure of the organ (Karakus et al., 2011; Shi et al., 2013).

Blood is very important for the life of human and domestic animals as it transfers the digestible nutrients, oxygen and trace elements from the gastrointestinal tract to all body tissue, and so it has a direct effect on body weight gain. It also shares in the elimination of cell metabolism into different pathways of biotransformation. The cells and biochemical parameter of the blood are affected by many diseases, heavy metals, pesticides, radiation, and drugs. Carbon tetrachloride was reported to induce anemia and leukocytosis in the blood as ensured by a marked decrease in RBC, PCV and Hb concentration (Famurewa et al., 2015).
Heart is a bio-vital organ that performed intense and continuous activity; therefore it has a less potent antioxidant system as compared with other organs (de Freitas et al., 2013). The endogenous protective mechanisms of it are unable to limit the excessive damaged caused by reactive oxygen species alone (Shi et al., 1993). The cardiac toxicity and congestive heart failure were also detected after CCL4 treatment (Plaa and Witschi, 1976). Supplementation of the body with natural antioxidant anti-inflammatory agent is essential to prevent the damage caused by oxidative stress and inflammatory mediators induced by carbon tetrachloride.

Numerous studies have displayed the antioxidant, anti-inflammatory and anti-hepatorenal toxicity effects of the extract of *Citrusus colocynthis* and *Moringa oleifera* (Nanjappaiah and Hugar, 2012; Sachan, 2012; Akhzari et al., 2015; Birendra et al., 2017; Pashmforosh et al., 2018; Mahmoud et al., 2019). In our previous studies, we demonstrated the hepatic and renal protective effect of the extract of *C. colocynthis* and *M. oleifera* against carbon tetrachloride toxicity in rats. The present study was conducted to investigate effect of extracts of tested plants on carbon tetrachloride induced hematological and cardiac toxicity in rats.

**MATERIALS AND METHODS**

**Medicinal plants**

*M. oleifera* leaves was extracted as described earlier (Fathy et al., 2017), while *C. colocynthis* fruits raw extract was extracted as described earlier (Mahmoud et al., 2019). The maximum ultraviolet absorbance (λmax) and construction of the calibration curves of both tested plants extracts were carried out inside the pharmaceutics laboratory, faculty of pharmacy, Mansoura University, Egypt, to ensure that each 1 g of the raw extract contained an exact 1 gm active principles to avoid the miss calculation of the administered doses of each plant (Mahmoud et al., 2019; Fathy et al., 2017; Mahmoud et al., 2019).

**Animals and treatments**

A total of 56 males’ albino rats weighed 130 to 150 g were kept in the laboratory under constant conditions of temperature (24 ± 2°C) for at least two week before and through the experimental work. All male albino rats were managed on standard nutritional diet (based on Nutrient Requirements of Laboratory Animals: Fourth Revised Edition, 1995) and water for ad-libitum. The animals were maintained in agreement with the guidelines prescribed by Ethics Committee of the Faculty of veterinary medicine, University of Mansoura, Egypt.

The experimental rats were divided into seven groups as shown in Table 1.

**Histopathological examination**

The treated animals and their controls were euthanized by over dose of thiopental after 8 weeks of treatment. Their heart was separated and fixed in 10% neutral buffered formalin. Fixed tissue were procedures were described previously before in details for eosin and hematoxylin staining (Bancroft and Gamble, 2002) and Mallory’s trichrome for collagen fibers detection (Drury and Wallington, 1980).

**Immunohistochemically localization of alpha smooth muscle actin (α-SMA) and interleukin-6 (IL-6)**

The heart paraffin sections that were mounted on positively charged glass slides were processed for single enzymatic immunohistochemistry with antibodies to alpha smooth muscle actin (α SMA) and interleukin-6 (IL-6). This protocol is carried out according to Karen et al. (2002). Briefly, tissue sections were deparaffinized and rehydrated using standard methods. Thereafter, these sections were incubated with hydrogen peroxide for 10 min (to block the endogenous peroxidase activity), then washed three times with phosphate buffer saline. Tissue sections were incubated for 2 h at 4°C with the 1st primary antibodies (α SMA(1:200) or IL-6 (1:100)) followed by washing with phosphate buffer saline. Secondary antibody (ready to use) (Biotinylated Goat Anti-Rabbit IgG, ab-64256, Abcam) was applied for 15 min, followed by washing with PBS. Each section was incubated with 200 μl of DAB solution for 10 min and was then washed with distilled water and immersed with Horseradish peroxidase (freshly prepared) until suitable staining develops (about 5 min). The sections were then rinsed in distilled water and counterstained with hematoxylin for 3 min. After that, they were washed, dehydrated, cleared, mounted and examined under light microscope.

**Morphometrical study**

The mean area percentage of collagen fiber content between the myocardocytes and around blood vessels was quantified for each group. The ten randomly sections stained with Mallory trichrome(x400) (for each group) were examined using image J analyzer program. The data that was obtained by this program was statistically analyzed and compared with the each other.

**Hematological examination**

Whole blood was collected on EDTA-vacuum tubes. Erythrocytes count and hemoglobin (Hb) was determined and then packed cell volume (PCV) was determined using conventional method. Erythrocytes indices as mean
Table 1: Experimental rats divided into seven groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tested chemicals</th>
<th>Dose</th>
<th>Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>olive oil (1/p)</td>
<td>0.5 ml</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Group2</td>
<td>CCL4 10% (1/p)</td>
<td>1 ml/kg</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Group3</td>
<td>Moringa (oral)</td>
<td>250 ml/kg</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Group4</td>
<td>CCL4 10% (1/p)</td>
<td>1 ml/kg</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Group5</td>
<td>Moringa (oral)</td>
<td>500 ml/kg</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Group6</td>
<td>Citrullus (oral)</td>
<td>12.5 mg/kg</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Group7</td>
<td>CCL4 10% (1/p)</td>
<td>1 ml/kg</td>
<td>8 weeks</td>
</tr>
<tr>
<td></td>
<td>Moringa (oral)</td>
<td>250 mg/kg</td>
<td>8 weeks</td>
</tr>
<tr>
<td></td>
<td>Citrullus (oral)</td>
<td>12.5 mg/kg</td>
<td>8 weeks</td>
</tr>
</tbody>
</table>

corpuscular values (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated. Total leukocytes and platelets count were also detected using automatic whole blood analyzer.

Biochemical assays

Sera were obtained by centrifugation of the blood samples for all rat groups and kept at 20°C until assayed for the biochemical markers: triglyceride level (Fossati, 1982), total cholesterol level (Fossati, 1982), sera total cholesterol level (Tietz, 1976), sera high density lipoprotein cholesterol (HDL-C) and Sera Low density lipoprotein cholesterol (LDL-C) levels (Tietz, 1976).

Electrophysiological (ECG) assessment of cardiac indices measured by BIOPAC

At end of treatment, rats were anesthetized by ketamine and follow all preparation and construction for cardiac indices measurement. At the start of the experimental protocol, ECG recording was done for all rats, then another ECG recording was done for all groups before scarification. ECG recording was done using BIOPAC student lab system (software BSL 3.7.5) in the Medical Physiology Department, Faculty of Medicine, Mansoura University. ECG leads were recorded through skin surface electrodes. The neutral electrode was connected to right hand leg, and right foreleg was connected to the negative electrode, while, left foreleg was connected to the positive electrode.

Statistical analysis

The all data in this study were statistically analyzed by one way ANOVA test (Snedecor and William, 1989). T-test was used to evaluate parametric and normalization of numerical data. Mean ± SDs were calculated with SPSS program (1996) version 13. p values <0.05 were considered as significant.

RESULTS

The prepared solutions were scanned spectrophotometrically at different wavelengths (200 – 400 nm) to determine the maximum wavelength (λmax) in order to confirm that each 1 g of the obtained extracts of M. oleifera and C. colocynthis contained an exact 1 g of the plants extract substance (Figure 1).

Hematological effect of single or combination of Moringa oleifera and Citrullus colocynthis on carbon tetrachloride induced injury

In the current study, it was found that only M. oleifera at dose of 500 mg/kg reduced significantly hemoglobin (Hb), red blood cells (RBCs), total leukocyte count (WBCs) and platelets count when compared with control group or carbon tetrachloride treated group. Moreover, M. oleifera at dose of 250 mg/kg reduced significantly platelets count and increased WBCs count significantly when compared with other groups, while carbon tetrachloride reduced non-
Figure 1: Concentration of extracts of Moringa oleifera and Citrullus colocynthis contained an exact 1 gm of the plants extract substance which was scanned spectrophotometrically at different wavelengths (200 – 400 nm) to determine the maximum wavelength (λmax).

Table 2: Hematological effect of single Moringa oleifera extract on carbon tetrachloride induced cardiomyopathy and hematotoxicity.

<table>
<thead>
<tr>
<th>Gr. 1</th>
<th>Hb (g/dL)</th>
<th>RBCs (10^6/µl)</th>
<th>HCT (%)</th>
<th>MCV (fl(µm³))</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>WBCs (10^3/µl)</th>
<th>Platelets (10^3/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr. 2</td>
<td>12.9±0.3</td>
<td>7.1±0.1</td>
<td>39.2±0.7</td>
<td>54.9±1.1</td>
<td>17.9±0.1</td>
<td>32.8±0.4</td>
<td>6.9±1.2</td>
<td>696±50</td>
</tr>
<tr>
<td>Gr. 2</td>
<td>13±0.3</td>
<td>6.8±0.26</td>
<td>39.9±0.7</td>
<td>58±0.1</td>
<td>18.8±0.2</td>
<td>32.5±0.3</td>
<td>9.9b1±2</td>
<td>776b1±48</td>
</tr>
<tr>
<td>Gr. 3</td>
<td>13±0.8</td>
<td>7.4±0.5</td>
<td>48.2±2.6</td>
<td>65±5</td>
<td>17.9±0.6</td>
<td>27.9±1.9</td>
<td>11.8b1±2.5</td>
<td>513b1,2±44</td>
</tr>
<tr>
<td>Gr. 4</td>
<td>12.2b±0.3</td>
<td>6.6b±0.26</td>
<td>42.8±1.4</td>
<td>64±3.7</td>
<td>18.3±0.2</td>
<td>28.5±1.4</td>
<td>5.6b±0.3</td>
<td>479b1,2±28</td>
</tr>
</tbody>
</table>

B1,2 significantly at ≤ 0.05.
Gr.1 control negative, Gr.2. received ccl4 only, Gr.3. received cd4+ Moringa oleifera at dose of 250 mg/kg, Gr. 4. received cd4+ Moringa oleifera at dose of 500 mg/kg.

Table 3: Hematological effect of single Citrullus colocynthis on carbon tetrachloride induced induced cardiomyopathy and hematotoxicity.

<table>
<thead>
<tr>
<th>Gr. 1</th>
<th>Hb (g/dL)</th>
<th>RBCs (10^6/µl)</th>
<th>HCT (%)</th>
<th>MCV (fl(µm³))</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>WBCs (10^3/µl)</th>
<th>Platelets (10^3/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr. 2</td>
<td>12.9±0.3</td>
<td>7.1±0.1</td>
<td>39.2±0.7</td>
<td>54.9±1.1</td>
<td>17.9±0.1</td>
<td>32.8±0.4</td>
<td>6.9±1.2</td>
<td>696±50</td>
</tr>
<tr>
<td>Gr. 2</td>
<td>13±0.3</td>
<td>6.8±0.26</td>
<td>39.9±0.7</td>
<td>58±0.1</td>
<td>18.8±0.2</td>
<td>32.5±0.3</td>
<td>9.9b1±2</td>
<td>776b1±48</td>
</tr>
<tr>
<td>Gr. 5</td>
<td>13±0.3</td>
<td>7.3±0.3</td>
<td>41.3±0.9</td>
<td>56.5±1</td>
<td>17.7±0.4</td>
<td>31.5±0.3</td>
<td>6.45±1.8</td>
<td>586b1±29</td>
</tr>
<tr>
<td>Gr. 6</td>
<td>12.9±0.5</td>
<td>7.2±0.4</td>
<td>52.7±0.3</td>
<td>58±2</td>
<td>17.9±0.3</td>
<td>30.9±0.7</td>
<td>6.46±0.5</td>
<td>493b1,2±38</td>
</tr>
</tbody>
</table>

B1,2 significantly at ≤ 0.05.
Gr.1 control negative, Gr.2. received ccl4 only, Gr.5. received cd4+ Citrullus colocynthis at dose of 12.5 mg/kg, Gr. 6. received dd4+ Citrullus colocynthis At dose of 25 mg/kg.

significantly level of RBCs, WBCs and platelets. In the current study, it was observed that only C. colocynthis at dose of 12.5 and 25 mg/kg reduced non-significantly total leukocyte count (WBCs) and platelets count when compared with control group or carbon tetrachloride treated group. Moreover, while carbon tetrachloride reduced non-significantly the level of red blood cells (RBCs), WBCs and platelets count. Additionally, the combined treatment of both plants increased non-significantly the blood indices in all parameters when compared with other groups (Tables 2 to 4).

Lipid profile parameters of single or combination of Moringa oleifera and Citrullus colocynthis on carbon tetrachloride induced injury

In the present study, carbon tetrachloride increased
The histological results

H&E stain

Group1: The light microscopic examination of the myocardial sections from this group (normal control) revealed the characteristic and classical histological structure of the myocardial cells. The longitudinal sections of these muscles appeared cylindrically branched and anastomosed with each other in a unique manner. This manner gave the appearance of a sheet with narrow spaces in between them (endomysium) (Figure 3a). The muscle fiber deeply stained striated acidophilic cytoplasm with central light vesicular oval nucleus. The endomysium between these individual muscle fibers contain blood capillaries and darkly stained elongated nuclei of the fibroblast cells (Figure 3b).

Group2: Examination of myocardial fibers from this group (CCl4) showed loss of normal histological structures of these fibers. Some fibers appeared wavy and widely separated from each other (Figure 3c). Most of the myocardial fibers had a dark pyknotic nucleus with loss of their cross striation. Some fibers showed area of sarcoplasmatic vaculation and others showed complete loss of their myofibrils or their nuclei (Figure 3c). A large numbers of different inflammatory cells and extravasated blood were detected between cardio myocytes (Figure 3d).

Lipid profile parameters of single or combination of Moringa oleifera and Citrullus colocynthis on carbon tetrachloride induced injury

Carbon tetrachloride was cardio-toxic as well as hemotoxic as described above and here evidence by abnormal ECG. Notably, single treatment of M. oleifera, Citrullus colocynthis or synergistic combined study of M. oleifera and C. colocynthis revealed ECG correction quite similar to control group (Figure 2 and Table 6).

Table 4: The hematological effect of combined treatment of Moringa oleifera and Citrullus colocynthis extract on carbon tetrachloride induced cardiomyopathy and hematotoxicity.

<table>
<thead>
<tr>
<th></th>
<th>Hb g/dL</th>
<th>RBCs 10⁶/µl</th>
<th>HCT %</th>
<th>MCV fl(μm³)</th>
<th>MCH pg</th>
<th>MCHC g/dL</th>
<th>WBCs 10³/µl</th>
<th>Platelets 10³/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr.1</td>
<td>12.9±0.3</td>
<td>7.1±0.1</td>
<td>39.2±0.7</td>
<td>54.9±1.1</td>
<td>17.9±0.1</td>
<td>32.8±0.4</td>
<td>6.9±1.2</td>
<td>696±50</td>
</tr>
<tr>
<td>Gr.2</td>
<td>13±0.3</td>
<td>6.8±0.26</td>
<td>39.9±0.7</td>
<td>58±0.1</td>
<td>18.8±0.2</td>
<td>32.5±0.3</td>
<td>9.9±1.2</td>
<td>776±48</td>
</tr>
<tr>
<td>Gr.7</td>
<td>13.6±0.7</td>
<td>7.4±0.24</td>
<td>44±1.4</td>
<td>59±2.2</td>
<td>18.1±0.4</td>
<td>30.7±0.4</td>
<td>7.3±2.2</td>
<td>745±46</td>
</tr>
</tbody>
</table>

B₁,₂ significantly at ≤ 0.05.
Gr.1 control negative, Gr.2 received CCl₄ only, Gr.7 received CCl₄+ Citrullus colocynthis at dose of 12.5 mg/kg + Moringa oleifera at dose of 250 mg/kg.

Table 5: Lipid profile parameters of single or combined Moringa oleifera and Citrullus colocynthis on carbon tetrachloride induced injury

<table>
<thead>
<tr>
<th></th>
<th>Triglyceride mg/dL</th>
<th>T. chol. mg/dL</th>
<th>LDL-C mg/dL</th>
<th>HDL-C mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr.1</td>
<td>35.4±6.5</td>
<td>57.76</td>
<td>29.9±6.3</td>
<td>23.2±3.1</td>
</tr>
<tr>
<td>Gr.2</td>
<td>43.8±3.4</td>
<td>42.2±6.1</td>
<td>8.2±4</td>
<td>25.4±2</td>
</tr>
<tr>
<td>Gr.3</td>
<td>15.2±0.85</td>
<td>25.7±1.9</td>
<td>9.3±2.2</td>
<td>13.2±3.4</td>
</tr>
<tr>
<td>Gr.4</td>
<td>26±3.6</td>
<td>30.7±4.3</td>
<td>17.7±2.6</td>
<td>7.7±0.8</td>
</tr>
<tr>
<td>Gr.5</td>
<td>14±2.2</td>
<td>29.5±4.1</td>
<td>13.7±3.5</td>
<td>13.1±2.3</td>
</tr>
<tr>
<td>Gr.6</td>
<td>12.2±11.9</td>
<td>28.2±2.3</td>
<td>14.1±3.4</td>
<td>11.7±1.1</td>
</tr>
<tr>
<td>Gr.7</td>
<td>17±4.9</td>
<td>23.2±2.9</td>
<td>8.2±2.4</td>
<td>11.6±1.02</td>
</tr>
</tbody>
</table>

B₁,₂ significantly at ≤ 0.05.
Gr.1 control negative, Gr.2 received CCl₄ only, Gr.3 received CCl₄+ Moringa oleifera at dose of 500 mg/kg, Gr.4 received CCl₄+ Citrullus colocynthis at dose of 12.5 mg/kg, Gr.5 received CCl₄+ Citrullus colocynthis at dose of 25 mg/kg.
Figure 2: Normal ECG of control negative a, abnormal ECG control positive b, while all plants treatment as *Moringa* at dose of 25 mg/kg c, *Citrullus colocynthis* at dose of 25 mg/kg d and synergistic plants e show quite normal ECG.

**Table 6**: All plants treatments were similar in improvement of heart indices especially HR (data concerning gr. 4,5,6 and 7 not shown to be quite similar).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>P-R (SEC)</th>
<th>QRS(mv)</th>
<th>QRS(sec)</th>
<th>QT(sec)</th>
<th>R-R(sec)</th>
<th>QTc</th>
<th>HR(Bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>control</td>
<td>0.043±0.001</td>
<td>0.31±0.021</td>
<td>0.037±0.0008</td>
<td>0.079±0.004</td>
<td>0.24±0.008</td>
<td>0.16±0.009</td>
<td>246.6±3.6</td>
</tr>
<tr>
<td>2</td>
<td>Ccl4</td>
<td>0.046±0.0001</td>
<td>0.33±0.021</td>
<td>0.041±0.0003</td>
<td>0.12±0.008</td>
<td>0.27±0.01</td>
<td>0.23±0.01</td>
<td>325±31</td>
</tr>
<tr>
<td>3</td>
<td>Ccl4+ Moringa 250 mg/kg</td>
<td>0.041±0.013</td>
<td>0.25±0.022</td>
<td>0.034±0.0006</td>
<td>0.23±0.14</td>
<td>0.24±0.01</td>
<td>0.16±0.01</td>
<td>255±14</td>
</tr>
<tr>
<td>4</td>
<td>Ccl4+ Moringa 500 mg/kg</td>
<td>0.0421±0.013</td>
<td>0.225±0.022</td>
<td>0.0324±0.0006</td>
<td>0.223±0.14</td>
<td>0.224±0.01</td>
<td>0.162±0.01</td>
<td>251.5±14</td>
</tr>
<tr>
<td>5</td>
<td>Ccl4+ Citrullus 12.5 mg/kg</td>
<td>0.0431±0.013</td>
<td>0.253±0.022</td>
<td>0.0343±0.0006</td>
<td>0.233±0.14</td>
<td>0.243±0.01</td>
<td>0.163±0.01</td>
<td>255.3±14</td>
</tr>
<tr>
<td>6</td>
<td>Ccl4+ Citrullus 25 mg/kg</td>
<td>0.0414±0.013</td>
<td>0.254±0.022</td>
<td>0.0344±0.0006</td>
<td>0.234±0.14</td>
<td>0.244±0.01</td>
<td>0.164±0.01</td>
<td>255.4±14</td>
</tr>
<tr>
<td>7</td>
<td>Ccl4+ Moringa 500 mg/kg+ Citrullus 12.5 mg/kg</td>
<td>0.0415±0.013</td>
<td>0.255±0.022</td>
<td>0.0345±0.0006</td>
<td>0.235±0.14</td>
<td>0.245±0.01</td>
<td>0.165±0.01</td>
<td>255.5±14</td>
</tr>
</tbody>
</table>

B¹² significantly at ≤ 0.05.

Cardiac myocytes. The cardiac myocytes had a central vesicular nucleus with acidophilic striated cytoplasm. The extravasated blood and the inflammatory cells nearly disappeared between the muscle fibers (Figure 3e and f).

**Mallory trichrome stain**

**Group 1**: The Mallory trichrome stained sections of this Group showed a few blue-stained collagen fibers in endomysium and around blood vessels (Figure 4a and b).

**Group 2**: The stained section of myocardiocytes showed that the amount of collagen fiber was markedly increased between the bundles of myocardiocytes as compared with the first group. Few area of a focal deposition of collagen fibers was also observed (Figure 4c and d).

**Group 3**: Minimal amount of collagen fibers nearly similar
Figure 3: Photomicrograph of myocardiocyte sections stained with H&E stain. (a,b): photomicrograph from the first group showing: cylindrical branched myocardiocytes with acidophilic cytoplasm (c) and vesicular light nucleus (curved arrow) separated with endomysium that contain blood vessels (thick arrow) and fibroblast (thick arrow). (c,d): photomicrograph of from the second group showing: (c): Wavy myocardiocytes with dark pyknotic nucleus (curved arrow) and widely separated from each other (asterisks) or infiltrated with mononucleated inflammatory cells (arrow). (d): Wide area with inflammatory cells infiltration. (e,f): photomicrograph from the third group showing: cylindrical branched cardiac muscle cells with light oval nucleus (curved arrow) separated with endomysium containing blood vessels (thick arrow) and fibroblast (thin arrow). (data concerning gr. 4,5,6 and 7 not shown as it quite similar).

Figure 4: Photomicrograph of myocardiocyte sections stained with Mallory trichrome stain. (a,b): photomicrograph from the first group showing: few amount of blue stained collagen fiber between myocardiocytes (arrows) or around blood vessels. (c,d): photomicrograph of from the second group showing: large amount of collagen fiber separated myocardiocytes (arrows) or completely displaced myocardiocytes (arrow head). (e,f): photomicrograph from the third group showing: few blue collagen fiber between cardiac muscle cells (arrow) or surrounded blood vessel (curved arrow). (data concerning gr. 4,5,6 and 7 not shown as it quite similar).
Enzymatic immunohistochemistry

Interleukin-6

Group 1: The positive immune reactivity of the interleukin-6 was not detected in the enzymatically stained sections of this group (Figure 5a and b).

Group 2: The strong positive brown reactivity of interleukin-6 was markedly observed between the cardiac muscle bundles in those areas that were observed infiltrated with the inflammatory cells in H&E stain (Figure 5c and d).

Group 3: These fifth treated groups with CCl4+250 /500 mg/kg b.w of M. oleifera or C. colocynthis or synergistic group exhibited the negative immune reactivity for IL-6 as the first control group (Figure 5e and f).

Alpha smooth muscle actin

Group 1: The positive brown immune reactivity of alpha smooth muscle actin of this group was only restricted to the smooth muscles that was present in the wall of blood vessels (Figure 6a and b).

Group 2: The brown immune reactive cells with alpha smooth muscle actin were observed in the wall of the blood vessels and within different areas displacing myocardiocytes (Figure 6c and d).

Group 3: The reactivity of alpha smooth muscle actin was only detected in the wall of the blood vessels as the first group. Also, the fifth treated groups with CCl4+250 /500 mg/kg b.w of M. oleifera or C. colocynthis or synergistic group exhibited the negative immune reactivity (Figure 6e and f).

The percentage area of collagen stained by Mallory trichrome

The mean percentage of collagen fiber density of the first three groups is shown in Table 7. The mean percentage of collagen fiber in the second group (11.5921b1.3±6893.3) was significantly increased as compared with the first group (4.9314 ±79639). Meanwhile, there was no significant difference between the first and third group or other plants treated groups (5.1964 ±55812).

DISCUSSION

Carbon tetrachloride increased significantly the levels of
triglycerides, while it reduced significantly the levels of low density lipoprotein cholesterol (LDL-C). *Citrus colocynthis* reduced significantly levels of lipid profile indices (Benmehdi et al., 2008) as *Citrus colocynthis* treatment decrease the elevated cholesterol and triglycerides in diabetic rats.

Also, *M. oleifera* at dose of 250 and 500 mg/kg when administered with carbon tetrachloride reduced lipid indices (Ouedraogo et al., 2013) as well as synergistic plants treatment with *M. oleifera* and *C. colocynthis*. Similarly, *M. oleifera* and gentamicin group showed a highly significant depletion in lipid peroxidation (LPO) level (Al-Malki and El Rabey, 2015).

Notably, in the presented study, combined treatment of *M. oleifera* at dose of 250 mg/kg and *C. colocynthis* at 12.5 mg/kg and in combination with carbon tetrachloride reduced the levels of lipid indices. Moreover, other study reported that *C. colocynthis* administration to diabetic rat improved dyslipidemia and attenuates the states of antioxidant enzyme and oxidative stress induced by diabetes mellitus (Omayma et al., 2013). In addition, the medicinal plant *C. colocynthis* fruit may have protective effect on tissues as it may play a role in preventing nephropathy as one of the microvascular complications of diabetes mellitus (El-Baky and Amin, 2011). Remarkably, the efficacy of the combined extracts of *Ocimum*

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**Figure 6:** Photomicrograph of myocardiocyte sections immune enzymatically stained with anti-alpha smooth muscle actin. (a,b) photomicrograph from the first group showing positive brown immune reactivity was detected only in wall of blood vessels (arrow). (c,d): photomicrograph of from the second group showing brown positive reaction was observed in the wall of blood vessels. (d) cells with brown positive immunoreaction were displaced myocardiocytes (arrow) and blood vessel was detected with positive reaction (curved arrow). (e,f): photomicrograph from the third group showing brown positive immune reaction was only restricted to the wall of blood vessels (arrow). (data concerning gr. 4,5,6 and 7 not shown as it quite similar).

**Table 7:** The mean percentage of collagen fiber density in plants treatment groups compared with control negative and positive one. (data concerning gr. 4,5,6 and 7 not shown as it quite similar).

<table>
<thead>
<tr>
<th>Collagen fiber density</th>
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<tbody>
<tr>
<td>Gr. 1</td>
<td>4.9314 ± 79639</td>
</tr>
<tr>
<td>Gr. 2</td>
<td>11.5921±.68933</td>
</tr>
<tr>
<td>Gr. 3</td>
<td>5.1964±.55812</td>
</tr>
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</table>

B^{1,2} significantly at ≤ 0.05.
gratissimum and M. oleifera ameliorates diabetic nephropathy complications at better manner in a synergistic pattern when compared with the single extracts and standard drugs insulin and glibenclamide (Iwara et al., 2011).

In the present study, the histopathological findings of cardiomyocytes after intraperitoneal injection of carbon tetrachloride showed: loss of their normal histological architecture as compared with the first group. In addition, the percentage area of collagen stained by Mallory trichrome was significantly increased as compared with the first group. A large numbers of inflammatory cells were also detected by hematoxylin and eosin.

Our results are in line with the previous studies that exhibited markedly pathological changes, as edema, and fibrosis and inflammation were detected in heart tissues of the rats after carbon tetra chloride injection (Eshaghi et al., 2012).

The current study also showed a strong positive immune reactivity for interleukin-6 as inflammatory cytokine in the group injected with carbon tetrachloride. This immune reactivity was not detected in the first group. The up regulation of the inflammatory cytokines after carbon tetrachloride may be attributed to the activation of the nuclear factor kappa-B, a proinflammatory transcription factor, that is an a critical step in the production of inflammatory cytokines such as IL-6 (Li et al., 2012). The critical role of IL-6 in heart dysfunction and infarction was confirmed by many previous studies (Irwin et al., 1999; De Ferranti and Rifai, 2007).

Alpha smooth muscle actin (α-SMA) is normally expressed during early stage of cardiac development. After that, it is replaced by alpha skeletal muscle actin (α-SKA) and alpha cardiac muscle actin (α-CA) (Suermeijer et al., 2003). The up regulation or reactivation of the expression of α-SMA reflected an important marker of cardiomyocytes failure or hypertrophy. (Friddle et al., 2000).

In the current study, the immune expression of α-SMA in normal heart was restricted to the smooth muscle that surrounded the blood vessels between cardiomyocytes. After carbon tetrachloride injection, the expression of α-SMA was detected around blood vessels and some positive brown immune reactive cells were also observed within different areas displacing cardiomyocytes. Our result is in line with the results published by (Leslie et al., 1991; Ingrid et al., 1994).

The improvement in the histological architecture of the cardiomyocytes following oral administration of M. oleifera and/or C. colocynthis was detected in the present study. No outstanding difference was found in the histological findings among Groups 3, 4, 5, 6 and 7, as protection study conducted against chronic injury induced by ccl4 and all plants in single or combined treatment showed antioxidant improvements.

The immune reactivity of the interleukin-6 or alpha smooth muscle actin and the percentage of collagen deposition between cardiomyocytes were nearly the same in the normal group. This improvement can be attributed to the antioxidant activities both of M. oleifera and C. colocynthis which has been demonstrated in numerous previous studies (Nanjappaiah and Hugar, 2012; Sachan, 2012; Akhzari et al., 2015; Birendra et al., 2017; Pasmathorosh et al., 2018; Mahmoud et al., 2019). The antioxidant activities of these plants were responsible for the down regulation in the level of the free radicals and the inflammatory cytokines.

Conclusions

Both M. oleifera and C. colocynthis raw extract in single or combined treatment improve soundness of heart architecture evidenced by the immunohistochemistry and reduction of lipid profile in the blood.

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